AS-924, a Novel Orally Active Bifunctional Prodrug of Ceftizoxime. Synthesis and Relationship between Physicochemical Properties and Oral Absorption

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Ceftizoxime (CZX), a parenteral cephalosporin, has potent and broad antibacterial activity. To improve its oral absorption, we synthesized a series of monofunctional and bifunctional prodrugs of CZX. In rabbits, urinary recovery after oral administration of CZX was improved by esterification of the carboxyl group at the C-4 position with various lipophilic moieties (monofunctional prodrugs), and was further increased by introduction of a hydrophilic L-alanine to the amino group on the thiazole ring at the C-7 position (bifunctional prodrugs). Least-squares analysis showed good parabolic correlations between log *P* and urinary recovery for monofunctional and bifunctional prodrugs, respectively. AS-924, a bifunctional prodrug with a pivaloyloxymethyl and L-alanyl moiety had the best balance of lipophilicity and water-solubility for oral absorption among the prodrugs synthesized.

Key words AS-924; ceftizoxime alapivoxil; cephalosporin; bifunctional produrg; lipophilicity; water-solubility

The so-called third generation parenteral cephalosporins bearing an aminothiazole–methoxyimino moiety at the C-7 position of the cephem nucleus, such as ceftizoxime (CZX), cefotaxime and cefmenoxime, possess a broad antibacterial spectrum and potent activity against gram-positive and gram-negative bacteria. However, they are poorly absorbed from the gastrointestinal tract because of their low lipophilicity due to the low pK_a value of the carboxyl group at the C-4 position of the cephem nucleus. In recent years, C-3 substituted cephalosporins bearing an aminothiazole–methoxyimino moiety at the C-7 position, including cefteram pivoxil (CFTM-PI)[1] and cefpodoxime proxetil (CPDX-PR),[2] have been developed as orally active prodrugs, in which the carboxyl group is esterified to increase lipophilicity. However, their antibacterial activities are still not satisfactory compared to the parenteral cephalosporins.

CZX, a parenteral cephalosporin with an aminothiazole–methoxyimino moiety, has a broad antibacterial spectrum against bacteria isolated from patients with respiratory or urinary tract infections, and more potent activity against gram-negative rods than oral cephalosporins used clinically.[3] It shows bactericidal activity at concentrations close to its minimum inhibitory concentrations (MIC) and is resistant to bacterial β-lactamases.[3] Moreover, CZX ester is assumed to be stable to isomerization from the Δ^3 to the Δ^2 ester because it has no substituent at the C-3 position.[4] Thus, CZX is considered to be a suitable parent compound for an orally active prodrug of cephalosporin exhibiting both potent antibacterial activity and good oral absorption. The drug administered orally as a solid is first dissolved in the gastrointestinal fluid, and then absorbed across the lipoidal intestinal membrane. Therefore, the drug needs to possess a good balance of water-solubility and lipophilicity to ensure good oral absorption and bioavailability. CZX has a weakly basic amino-thiazole moiety on its side chain at the C-7 position (pK_a 2.95), and esterification alone may result in an increase of lipophilicity accompanied by reduction of water-solubility needed for dissolution into the gastrointestinal fluid. We previously resolved this problem by introduction of a hydrophilic moiety such as an amino acid into the ester of cephalosporin.[5—7]

In the present study, to further increase the oral absorption of CZX esters (monofunctional prodrugs) by improvement of water-solubility, we introduced various amino acids as basic moieties onto the weakly basic amino group on the thiazole ring (bifunctional prodrugs). Then, we examined the correlation between oral absorption and physicochemical properties for monofunctional and bifunctional prodrugs. Among the bifunctional prodrugs examined, pivaloyloxymethyl 7β-[(*Z*)-2-(2-(*S*)-alanylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate hydrochloride (ceftizoxime alapivoxil, AS-924) was demonstrated to have the best balance of lipophilicity and water-solubility for oral absorption (Fig.1).

Chemistry The synthetic routes of the monofunctional prodrug compounds **2a**—**2f**, esterified with various lipophilic moieties at the C-4 carboxyl group of CZX, are shown in Chart 1. The free acid compound of CZX was treated with iodomethyl pivalate in *N*,*N*-dimethylacetamide (DMAc) in the presence of dicyclohexylamine (DCHA) to afford the ester compound **2a**. The other monofunctional prodrugs **2b**—**2f** were prepared by esterification of CZX with the corresponding halides under the same reaction conditions.

The synthetic routes of the bifunctional prodrugs **4g**—**4p**, in which various amino acids were introduced into the monofunctional prodrugs, are shown in Chart 2. The ester compound **2a** was treated with *N*-*tert*-butoxycarbonyl(*N*-Boc)-L-alanine in methylene chloride in the presence of 4-dimethylaminopyridine (DMAP), using 1-ethyl-3-(3-diethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) as a condensing agent to afford compound **3h**. Finally, the *N*-Boc group of compound **3h** was removed by treatment with hydrochloride in formic acid to afford compound **4h** (AS-924). The other bifunctional prodrugs **4g**, **4i**—**4p** were prepared by the same procedure from the corresponding monofunctional

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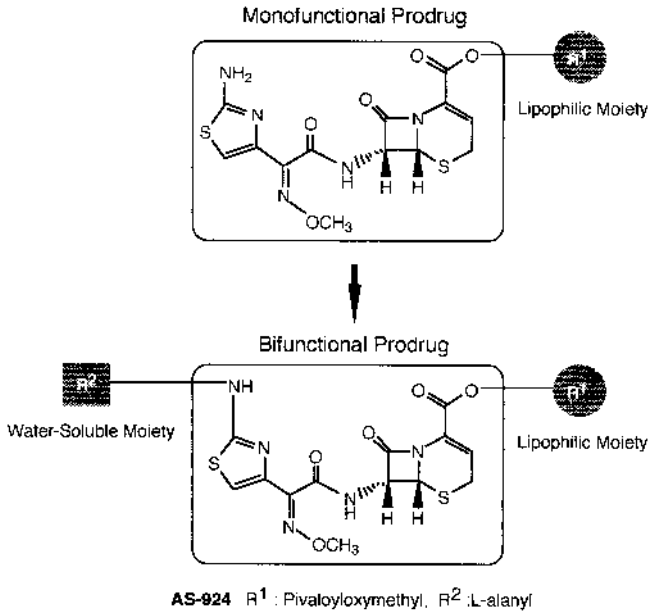


Fig. 1. Chemical Structures of Prodrugs of CZX

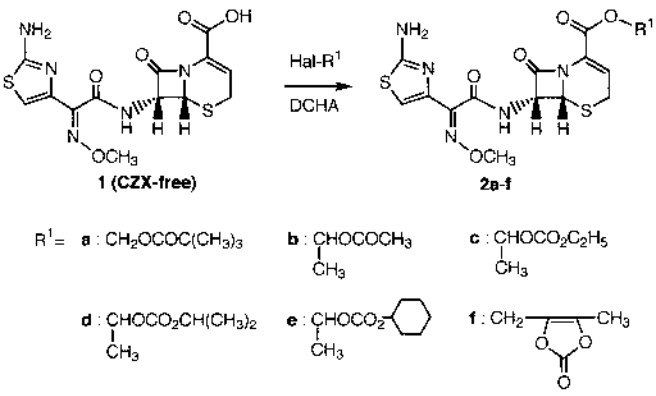


Chart 1

prodrugs.

The synthetic routes for compounds **7a**—**7f**, in which various amino acids were introduced onto the amino group of the thiazole ring of CZX, are shown in Chart 3. Compounds **7a**—**7f** were prepared using the diphenylmethyl ester of CZX (**5**) as the starting material under reaction conditions similar to those shown in Chart 2.

Biological Results

Oral Absorption and Physicochemical Properties of Monofunctional Prodrugs A series of monofunctional prodrugs (**2a**—**2f**) was synthesized by esterification of the C-4 carboxyl group of CZX with various lipophilic moieties. Physicochemical properties and urinary recovery (UR) after oral administration of the prodrugs in rabbits were determined and are summarized in Table 1. The UR was markedly increased by esterification from 5.36% for CZX to 11.12—31.59%. Among the monofunctional prodrugs synthesized, the pivaloyloxymethyl (POM) ester of CZX (**2a**) showed the highest UR. Least-squares analysis showed a parabolic relationship between $\log P$ and UR values for the monofunctional prodrugs with an optimum $\log P$ value of 1.81 (Fig. 2).

$$\mathrm{UR} = -6.730\,(\log P)^2 + 24.333\log P + 10.469 \quad (r^2 = 0.864)$$

Water-solubility values for the synthesized compounds at pH 4.5 and 6.0, which are the virtual pH on the surface of small intestinal mucosa,[8a,b] were determined and are summarized in Table 1. Compounds **2a**, **2d** and **2e** with relatively high $\log P$ values were poorly soluble in water, indicating that oral absorption of these prodrugs might be further increased by improvement of the water-solubility.

Oral Absorption and Physicochemical Properties of Bifunctional Prodrugs To increase water-solubility, various amino acids were introduced as basic moieties onto the weakly basic amino group on the thiazole ring of the POM ester of CZX (**2a**), which showed the best oral absorption among the monofunctional prodrugs examined as described

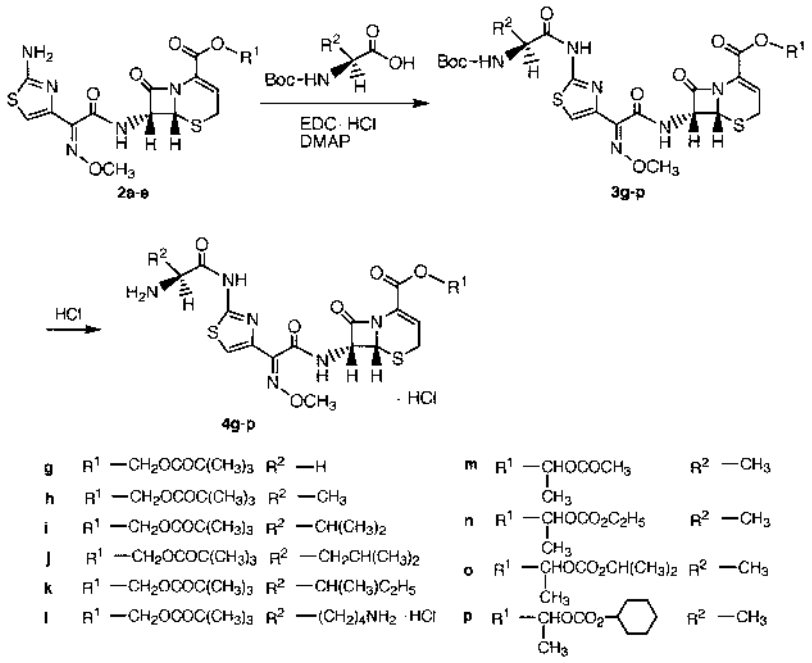


Chart 2

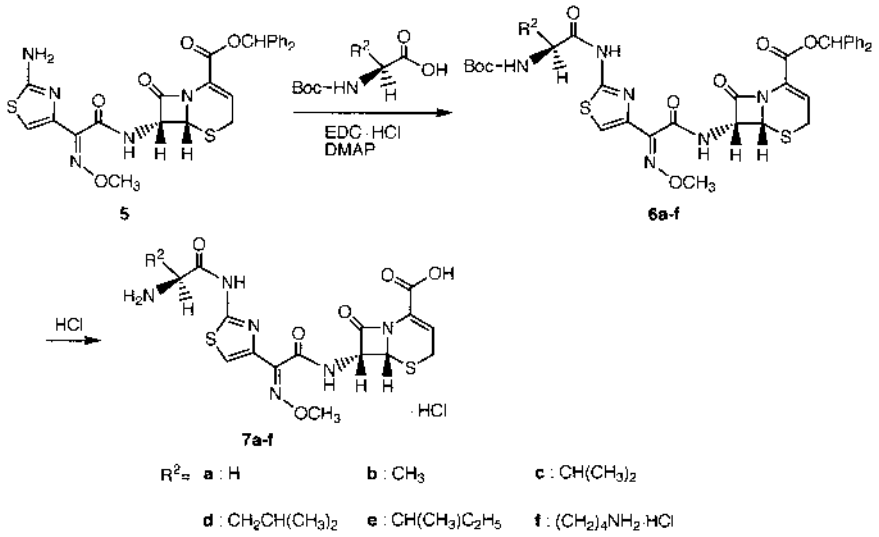
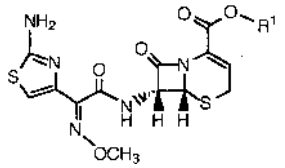


Chart 3

Table 1. Physicochemical Properties and Oral Absorption in Rabbits of Monofunctional Prodrugs of CZX



Compound No.	R^1	log P[a]	Water-solubility[b] (mg/ml)		UR[c] (%)[d]
			pH 4.5	pH 6.0	
CZX	—Na	−3.29	>25	>25	5.36±1.62
2a	$-CH_2OCOC(CH_3)_3$	1.57	0.42	0.38	31.59±1.51
2b	$-CHOCOCH_3$ (CH3)	0.46	2.08	1.72	25.21±3.00
2c	$-CHOCO_2C_2H_5$ (CH3)	0.96	0.80	0.58	27.42±1.89
2d	$-CHOCO_2CH(CH_3)_2$ (CH3)	1.37	0.43	0.30	29.68±3.54
2e	$-CHOCO_2$-cyclohexyl (CH3)	2.52	0.03	0.04	29.56±2.29
2f	$-CH_2$-(5-methyl-2-oxo-1,3-dioxol-4-yl)	0.17	0.11	0.19	11.12±1.75

a) 1/15 M phosphate buffer (pH 6.5)/*n*-octanol. *b*) 1/15 M phosphate buffer. *c*) Urine was collected for 6 h after oral administration. *d*) Each value represents the mean± S.D. of three animals.

above. Introduction of amino acids to **2a** markedly increased the water-solubility at both pH 4.5 and 6.0. However, UR was increased only in the L-alanyl derivative **4h** (AS-924), indicating that the L-alanyl moiety is the most effective amino acid residue for bifunctional prodrugs of CZX (Table 2).

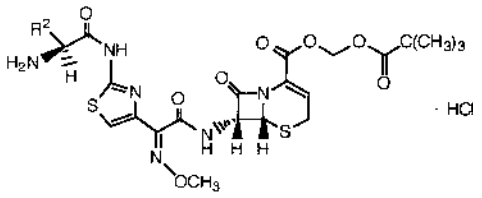
In the next set of experiments, we introduced L-alanine into various esters of CZX. Their physicochemical properties and UR are shown in Table 3. The apparent log P values of bifunctional prodrugs **4m**—**4p** were calculated from the relation between **2a** and AS-924. Introduction of L-alanine into CZX esters increased the water-solubility and decreased the log P value to a similar extent. However, apparent log P values for bifunctional prodrugs **4m**—**4p** were still higher than that of CZX. UR of the bifunctional prodrugs **4m**—**4p** were also higher than those of corresponding monofunctional prodrugs **2b**—**2e**. For the bifunctional prodrugs, a parabolic correlation between log P and UR was obtained with an optimum log P value of 1.22 (Fig. 2).

$$UR = -8.170\,(\log P)^2 + 19.974 \log P + 28.342 \quad (r^2 = 0.851)$$

Figure 2 clearly shows that oral absorption of all the monofunctional prodrugs was increased by introduction of an L-alanyl moiety (bifunctional prodrugs).

Hydrolysis of CZX with Various Amino Acids Prodrugs are required to be stable in the gastrointestinal lumen and to be rapidly converted to the parent drugs during and/or after absorption. Esters of cephalosporins at the C-4 position are known to be rapidly hydrolyzed by intestinal esterase.

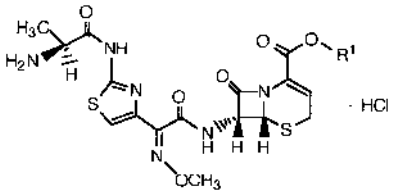
Table 2. Physicochemical Properties and Oral Absorption in Rabbits of Bifunctional Prodrugs of CZX



Compound No.	R^2	log $P^{a)}$	Water-solubility$^{b)}$ (mg/ml)		UR$^{c)}$ (%)$^{d)}$
			pH 4.5	pH 6.0	
4g	–H	0.50	7.07	2.51	17.65±1.49
4h (AS-924)	$-CH_3$	1.15	19.9	4.32	42.82±2.63
4i	$-CH(CH_3)_2$	1.98	>25	6.67	16.11±3.35
4j	$-CH_2CH(CH_3)_2$	2.95	>25	4.19	28.76±3.27
4k	$-CH(CH_3)C_2H_5$	3.28	>25	5.05	19.63±2.74
4l	$-(CH_2)_4NH_2\cdot HCl$	−0.37	>25	8.91	21.02±3.18

a) 1/15 M phosphate buffer (pH 6.5)/*n*-octanol. *b*) 1/15 M phosphate buffer. *c*) Urine was collected for 6 h after oral administration. *d*) Each value represents the mean±S.D. of three animals.

Table 3. Physicochemical Properties and Oral Absorption in Rabbits of Bifunctional Prodrugs of CZX



Compound No.	R^1	log $P^{a)}$	Water-solubility$^{b)}$ (mg/ml)		UR$^{c)}$ (%)$^{d)}$
			pH 4.5	pH 6.0	
4h (AS-924)	$-CH_2OCOC(CH_3)_3$	1.15	19.9	4.32	42.82±2.63
4m	$-CHOCOCH_3$ (CH$_3$)	0.04	>25	>25	29.48±1.31
4n	$-CHOCO_2C_2H_5$ (CH$_3$)	0.54	18.5	5.77	36.40±1.90
4o	$-CHOCO_2CH(CH_3)_2$ (CH$_3$)	0.95	>25	5.28	37.87±1.90
4p	$-CHOCO_2-C_6H_{11}$ (CH$_3$)	2.10	>25	0.40	34.01±2.29

a) Estimated from the relation between **2a** and **4h**. *b*) 1/15 M phosphate buffer. *c*) Urine was collected for 6 h after oral administration. *d*) Each value represents the mean±S.D. of three animals.

However, little is known about hydrolysis of the amide bond between amino acids and amino moieties on the thiazole ring at the C-7 position of cephalosporins. Thus, hydrolysis of CZX with various amino acid moieties and release of CZX were examined in rabbit intestinal homogenate (Fig. 3). Hydrolysis of the CZX amides varied according to the amino acid species introduced, with half-lives time ranging from 5.90 to 79.47 min. The L-valine derivative (**7c**) was hydrolyzed most slowly, and more than 60% remained after incubation for 60 min at 37 °C, while the L-leucine (**7d**) and L-lysine derivatives (**7f**) were almost completely degraded within 30 min. The L-alanine (**7b**) derivative disappeared over 60 min. The disappearance of CZX derivatives with various amino acid moieties in the intestinal homogenate showed the same time course as the appearance of CZX.

Oral Absorption of 4h (AS-924) and Other Cephalosporin Esters In rabbits, oral absorption of AS-924 was compared with other orally active cephalosporin prodrugs used clinically. The UR of AS-924, CFTM-PI, CPDX-PR and cefotiam hexetil (CTM-HE) were 42.82±2.63, 27.41±6.06, 55.62±8.20 and 28.77±2.72% (mean±S.D.), respectively, after oral administration at 20 mg/kg as an active compound. AS-924 was shown to be orally absorbed more efficiently than CFTM-PI and CTM-HE. The UR of CPDX-PR was higher than that of AS-924.

Oral Absorption of a Monofunctional and Bifunctional Prodrug of CZX Administered Using a Capsule In dogs, the values of C_{max}, T_{max} and area under the plasma concentration (AUC_{0-8}) of CZX after oral administration of AS-924, a bifunctional prodrug and **2a**, a monofunctional prodrug filled in a capsule was determined (Table 4). The C_{max}, T_{max} and AUC_{0-8} of CZX after the administration of AS-924 at a dose

of 10 mg/kg as an active compound were 3.41±0.36 μg/ml, 1.71±0.40 h and 10.38±0.98 μg·h/ml, while those of **2a** were 1.80±0.68 μg/ml, 2.25±1.89 h and 5.38±2.57 μg·h/ml (mean±S.D.). The C_{max} and AUC_{0-8} of CZX after oral administration of AS-924 was about two times higher than that of **2a**.

Discussion

In general, β-lactam antibiotics are poorly absorbed from the gastrointestinal tract because of their low lipophilicity due to the low pK_a value of the carboxyl group. There have been some reports of successful prodrug design by esterification of the carboxyl groups in penicillin and cephalosporin derivatives.[2,3,9—14] In the present study, a series of monofunctional prodrugs of CZX was prepared by esterification with various lipophilic pro-moieties at the C-4 position. The lipophilicity of CZX determined as log P (−3.29) was markedly increased by esterification in monofunctional prodrugs (0.17—2.52). Compounds **2a**, **2d** and **2e** with log P values of 1.37—2.52 showed relatively high UR, and **2f** with the lowest log P value 0.17 showed the lowest UR. The UR values showed a parabolic relationship against log P values as reported for other cephalosporins.[15a,b] The POM ester of CZX (**2a**) showed the best UR among the monofunctional prodrugs examined and had a nearly optimum log P value of 1.57 for oral absorption. A log P value favorable for oral absorption is generally considered to be about 2.0,[16] and has been reported to be 1.23—2.14 for cephalosporins with an esterified moiety at the C-7 position,[15a] and 1.52—2.67 for POM esters of C-3 substituted cephalosporins.[15b]

However, oral absorption of compounds with relatively high log P values may be limited by low water-solubility insufficient for dissolution in intestinal fluid. In cephalosporins with a neutral or weakly basic moiety at the C-7 position, esterification at the C-4 position may not improve the oral absorption because of reduction of water-solubility. It has been reported that oral absorption of cephalosporin prodrugs is dependent on water-solubility, if the lipophilicity and hydrolysis rate are sufficiently high.[8a] To improve oral absorption of esterified prodrugs of cephalosporins, we increased the water-solubility by introduction of a glycyl moiety onto the ester moiety at the C-4 position[17] and an L-alanyl moiety onto the hydroxyl group of the side chain at the C-7 position.[5—7] Among the monofunctional prodrugs synthesized, compound **2a** showed the best log P and UR but was hardly soluble in water, indicating that absorption can be further increased by improvement of its water-solubility. Therefore, various amino acids as basic moieties were introduced

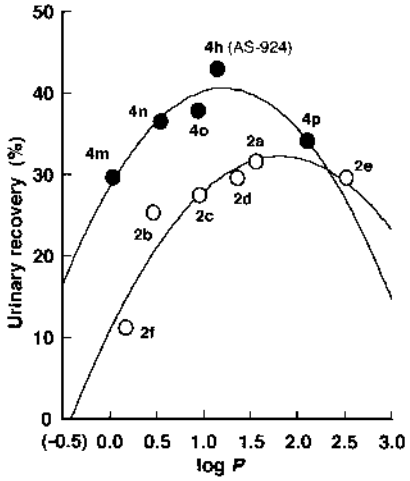


Fig. 2. Relationship between Lipophilicity (log P) and the UR of Monofunctional and Bifunctional Prodrugs of CZX

Table 4. Pharmacokinetic Parameters after Oral Administration of AS-924 and **2a** in Capsules at a Dose of 10 mg/kg in Dogs

Drug	C_{max} [a] (μg/ml)	T_{max} [a] (h)	AUC_{0-8} [a] (μg·h/ml)
AS-924	3.41±0.36	1.71±0.40	10.38±0.98
2a	1.80±0.68	2.25±1.89	5.38±2.57

a) Each value represents the mean±S.D. of six animals.

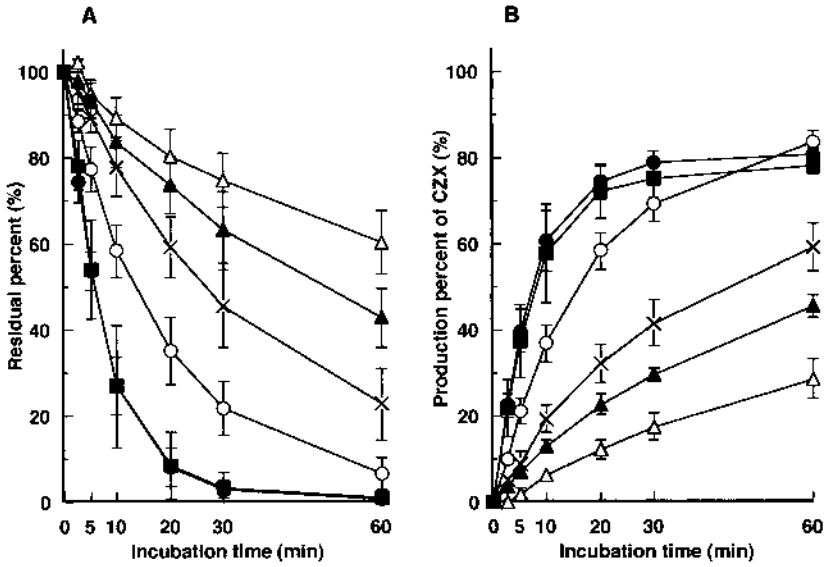


Fig. 3. Hydrolysis of CZX with Various Amino Acid Moieties in Rabbit Small Intestine Homogenate

A: Decrease of CZX with various amino acid moieties, B: Production of CZX, mean±S.D., n=3. Each compound (final concentration: 20 μg/ml) was incubated with 5% homogenate of small intestine of rabbits at 37 °C. —▲—: **7a**, —○—: **7b**, —△—: **7c**, —●—: **7d**, —×—: **7e**, —■—: **7f**.

onto the weakly basic amino group at the C-7 position in **2a**, a POM ester of CZX. AS-924, a bifunctional prodrug of CZX with an L-alanyl and POM moiety, showed a good log *P* value for absorption and the highest oral absorption among the prodrugs with other amino acids. The log *P* value was too low in compounds with glycyl and L-lysyl moieties (**4g**, **4l**), and too high in compounds with L-leucyl and L-isoleucyl moieties (**4j**, **4k**). In the CZX ester with a L-valyl moiety (**4i**), oral absorption was not increased despite an appropriate log *P* value and water-solubility. Therefore, factors other than lipophilicity and water-solubility appeared to influence its absorption. Prodrugs are required to be converted to their parent compounds at the appropriate place and time during and/or after absorption. Compound **4i** was resistant to hydrolysis in rabbit intestinal homogenate.

The UR values of the bifunctional prodrugs, in which L-alanine was introduced to the various esters of CZX showed a parabolic correlation to log *P*. The log *P* value of AS-924 was closest to the calculated optimal values for absorption among the prodrugs examined. At all log *P* values, the UR values of bifunctional prodrugs were higher than those of the corresponding monofunctional prodrug, clearly indicating that introduction of an L-alanyl moiety improved the oral absorption by increasing the water-solubility (Fig. 2).

In rabbits, AS-924 showed a higher UR value after oral administration than CFTM-PI and CTM-HE, both orally active cephalosporins used clinically. Drugs given orally as a form of tablets or capsules are absorbed *via* disintegration and dissolution. Thus, the water-solubility of the drug is an important factor in oral absorption when drugs are administered to patients as pharmaceutical dosage forms. Indeed, an improvement in oral absorption of the CZX ester by the L-alanyl moiety was more clearly demonstrated when administered using capsule in dogs (Table 4).

In conclusion, a bifunctional prodrug method using an L-alanyl moiety was demonstrated to be applicable to parenteral cephalosporins bearing an aminothiazole–oxime moiety such as CZX. AS-924, a bifunctional prodrug of CZX with an L-alanyl and POM moiety showed a good balance of lipophilicity and water-solubility for oral absorption, and is expected to be efficiently and reliably absorbed when administered clinically as a pharmaceutical dosage form.

Experimental

IR spectra were taken with a JASCO IR-800 spectrometer. ^{1}H-NMR spectra were recorded at 60 or 90 MHz on a Hitachi R-600 or R-1900 spectrometer using tetramethylsilane (TMS) as an internal standard. For column chromatography, silica gel (Daisogel No.1001W, Daiso) was used.

Antibiotics AS-924 and its related compounds were prepared in our Research Laboratories. CZX, CFTM-PI, CPDX-PR and CTM-HE were obtained commercially.

Water-Solubility Water-solubility was determined according to Yoshimura *et al.*[8a] Each compound (25 mg) was added to 1/15 M phosphate buffer (2.0 ml) of pH 4.5 or 6.0, and the mixture was shaken vigorously for 30 min at room temperature. After the mixture was filtered through a membrane filter with a pore size of 0.45 μm, the concentration of each compound was measured by HPLC.

Partition Coefficient Partition coefficients of CZX or compounds **2a**—**2f** were measured by the flask-shaking method. Phosphate buffer (1/15 M, pH 6.5), or *n*-octanol solution of CZX or compound **2a**—**2f** (*ca.* 500 μg/ml) was shaken vigorously for 60 s at room temperature with the same volume of phosphate buffer or *n*-octanol. After centrifugation for 5 min at 2500 r.p.m., the concentrations of CZX or compound **2a**—**2f** in both phases were measured by HPLC. Each solvent (buffer, *n*-octanol) used was saturated with the other solvent before use. The partition coefficients of compounds **4g**—**4l** were determined by HPLC as described by Toon *et al.*[18]

Oral Absorption Study in Rabbits Male *albino* rabbits, weighing 2.0—3.4 kg, were fasted overnight before dosing but were given free access to water. The test compounds were given orally at a dose of 20 mg/kg as CZX dissolved in distilled water or suspended in 0.5% methyl cellulose solution using an oral tube. The oral tube after administration of the test compound was flushed with distilled water (2 ml). Urine was collected through a ureteral catheter from 0 to 3 and 3 to 6 h. The concentration of CZX in urine was measured by the disc-plate diffusion method using *Escherichia coli* ATCC 39188 as the test organism and nutrient agar (Difco) as the test medium.

Oral Absorption Study in Dog Male beagle dog weighing 10—13 kg were used after fasting overnight prior to dosing. Compound **2a** or AS-924 filled in gelatin capsule (No. 1) was given orally at a dose of 10 mg/kg as CZX and 20 ml of water was given through the oral tube. Blood was taken from the cephalic vein of foreleg of the dog at specified times and centrifuged for 10 min. The concentration of CZX in plasma was measured by the agar-well diffusion method similar to the method mentioned above.

Hydrolysis Male *albino* rabbits, fasted, but given free access to water for 16—18 h before the experiments, were sacrificed. The small intestine was removed immediately, washed several times with ice-cooled saline to expel the luminal contents, and homogenized with 3 volumes of 1/15 M phosphate buffer (pH 7.0). After centrifugation at 12000 r.p.m. for 20 min at 0 °C, the supernatant was diluted with 4 volumes of 1/15 M phosphate buffer, and this solution was used as a 5% small intestine homogenate.

Various amino acid derivatives of CZX as substrates were dissolved in *N*,*N*-dimethylformamide at a concentration equivalent to 2 mg/ml of CZX. The solution (5 μl) was rapidly added to the 5% small intestine homogenate (0.5 ml) preheated at 37 °C so that the final concentration of the substrate was equivalent to 20 μg/ml of CZX. Sampling was carried out after 2.5, 5, 10, 20, 30 and 60 min of incubation at 37 °C. The sample was added to the same volume of a mixture of 6% trichloroacetic acid–acetonitrile (1 : 1), and was shaken vigorously. After centrifugation at 15000 r.p.m. for 5 min at 0 °C, the concentration of each substrate and CZX in the supernatant was measured by HPLC.

Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (2a) To a suspension of the free acid of CZX (9.14 g) in DMAc (45 ml) was added DCHA (5.22 ml) and iodomethyl pivalate (7.5 g) at −5 °C. After stirring for 2 h at the same temperature, EtOAc (100 ml) was added and the resulting precipitate was filtered off. The filtrate was washed successively with aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene–EtOAc, 1 : 1→1 : 2) to give **2a** (8.66 g) in 73% yield. IR (Nujol) cm^{-1}: 1785, 1750, 1680. ^{1}H-NMR (DMSO-d_6) δ: 1.16 (s, 9H), 3.50—3.80 (m, 2H), 3.84 (s, 3H), 5.13 (d, 1H, J=5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.70 (m, 1H), 6.73 (s, 1H), 7.12 (br, 2H), 9.67 (d, 1H, J=8.0 Hz).

The ester compounds **2b**—**2f** were prepared by a procedure similar to that described above. IR and ^{1}H-NMR spectral data are listed in Table 5.

Pivaloyloxymethyl 7β-[(*Z*)-2-[2-*N*-(*tert*-Butoxycarbonyl)-(*S*)-alanylaminothiazol-4-yl)]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (3h) To a solution of **2a** (9.6 g) and *N*-Boc-L-alanine (7.8 g) in methylene chloride (100 ml) was added EDC·HCl (7.9 g) and DMAP (0.23 g) at room temperature. After stirring for 2 h, the reaction mixture was washed with 10% aq. citric acid, 5% aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (Daisogel No. 1001 W, eluent; Benzene–EtOAc, 3 : 1→2 : 1) to give **3h** (6.45 g) in 50% yield. IR (Nujol) cm^{-1}: 1780, 1755, 1680. ^{1}H-NMR (DMSO-d_6) δ: 1.16 (s, 9H), 1.27 (d, 3H, J=6.0 Hz), 1.36 (s, 9H), 3.50—3.80 (m, 2H), 3.89 (s, 3H), 3.90—4.40 (m, 1H), 5.14 (d, 1H, J=5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.80 (m, 1H), 7.05 (d, 1H, J=5.0 Hz), 7.30 (s, 1H), 9.17 (d, 1H, J=8.0 Hz), 12.4 (br s, 1H).

Compounds **3g**, **3i**—**3p** were prepared by a procedure similar to that described above. IR and ^{1}H-NMR spectral data are listed in Table 6.

Pivaloyloxymethyl 7β-[(*Z*)-2-[2-(*S*)-Alanylaminothiazol-4-yl)]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate Hydrochloride (4h, AS-924) To a solution of **3h** (4.9 g) in formic acid (25 ml) was added 9.15 N HCl–isopropanol (3.2 ml) at 7 °C with stirring. After stirring for 5 min at 0—5 °C, the reaction mixture was poured into diethyl ether (100 ml). The resulting precipitate was collected by filtration. The solid was dissolved in methanol and poured into diisopropyl ether to give AS-924 (4.08 g) as a powder in 92% yield. IR (Nujol) cm^{-1}: 1775, 1750, 1670. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (s, 9H), 1.52 (d, 3H, J=6.0 Hz), 3.50—3.90 (m, 2H),

Table 5. ^{1}H-NMR and IR Spectral Data (**2b**—**2f**)

Compound No.	^{1}H-NMR(60 MHz, DMSO-d_6, δ)	IR (Nujol) cm^{-1}
2b	1.47 (3H, d, J=6.0 Hz), 2.05 (3H, s), 3.50—3.80 (2H, m), 3.84 (3H, s), 5.12 (1H, d, J=5.0 Hz), 5.84 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.73 (1H, s), 6.88 (1H, q, J=6.0 Hz), 7.17 (2H, br), 9.62 (1H, d, J=8.0 Hz)	1770, 1675
2c	1.22 (3H, d, J=7.0 Hz), 1.50 (3H, d, J=6.0 Hz), 3.50—3.80 (2H, m), 3.84 (3H, s), 4.16 (2H, q, J=6.0 Hz), 5.13 (1H, d, J=5.0 Hz), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.73 (1H, s), 6.70—7.00 (1H, m), 7.00—7.60 (2H, br), 9.57 (1H, d, J=8.0 Hz)	1770, 1675
2d	1.24 (6H, d, J=6.0 Hz), 1.49 (3H, d, J=6.0 Hz), 3.50—3.80 (2H, m), 3.84 (3H, s), 4.60—5.00 (1H, m), 5.13 (1H, d, J=5.0 Hz), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.70—7.00 (1H, m), 6.73 (1H, s), 7.00—7.60 (2H, br), 9.57 (1H, d, J=8.0 Hz)	1760, 1675
2e	1.00—2.10 (10H, m), 1.50 (3H, d, J=6.0 Hz), 3.50—3.70 (2H, m), 3.84 (3H, s), 4.30—4.70 (1H, m), 5.13 (1H, d, J=5.0 Hz), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.72 (1H, s), 6.70—7.00 (1H, m), 7.18 (2H, br s), 9.57 (1H, d, J=8.0 Hz)	1760, 1675
2f	2.18 (3H, s), 3.50—3.80 (2H, m), 3.84 (3H, s), 5.00—5.30 (1H, m), 5.15 (1H, s), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.73 (1H, s), 7.17 (2H, br s), 9.57 (1H, d, J=8.0 Hz)	1820, 1780, 1735, 1675

Table 6. ^{1}H-NMR and IR Spectral Data (**2b**—**2f**)

Compound No.	^{1}H-NMR(60 MHz, DMSO-d_6, δ)	IR (Nujol) cm^{-1}
3g	1.16 (9H, s), 1.39 (9H, s), 3.50—3.80 (2H, m), 3.70—4.10 (2H, m), 3.89 (3H, s), 5.15 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 6.90—7.20 (1H, m), 7.35 (1H, s), 9.66 (1H, d, J=8.0 Hz), 12.50 (1H, br s)	1770, 1675
3i	0.88 (6H, d, J=5.0 Hz), 1.16 (9H, s), 1.37 (9H, s), 1.70—2.20 (1H, m), 3.50—3.80 (2H, m), 3.89 (3H, s), 3.90—4.20 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 6.93 (1H, d, J=9.0 Hz), 7.35 (1H, s), 9.67 (1H, d, J=8.0 Hz), 12.50 (1H, br s)	1770, 1675
3j	0.89 (6H, d, J=6.0 Hz), 1.16 (9H, s), 1.50—1.90 (3H, m), 1.36 (9H, s), 3.50—3.80 (2H, m), 3.90 (3H, s), 4.00—4.40 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.03 (1H, d, J=9.0 Hz), 7.34 (1H, s), 9.68 (1H, d, J=8.0 Hz), 12.54 (1H, br s)	1760, 1675
3k	0.70—1.10 (6H, m), 1.16 (9H, s), 1.10—2.00 (3H, m), 1.36 (9H, s), 3.50—3.80 (2H, m), 3.89 (3H, s), 4.00—4.30 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.03 (1H, d, J=9.0 Hz), 7.35 (1H, s), 9.68 (1H, d, J=8.0 Hz), 12.49 (1H, br s)	1790, 1755, 1680
3l	1.16 (9H, s), 1.20—1.90 (6H, m), 1.37 (18H, s), 2.70—3.10 (2H, m), 3.50—3.80 (2H, m), 3.89 (3H, s), 4.00—4.30 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.80 (2H, m), 7.02 (1H, d, J=9.0 Hz), 7.35 (1H, s), 9.67 (1H, d, J=8.0 Hz), 12.51 (1H, br s)	1735, 1675
3m	1.27 (3H, d, J=5.5 Hz), 1.36 (9H, s), 1.48 (3H, d, J=5.5 Hz), 2.05 (3H, s), 3.50—3.70 (2H, m), 3.90—4.40 (1H, m), 3.90 (3H, s), 5.15 (1H, d, J=5.0 Hz), 5.89 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.88 (1H, q, J=5.5 Hz), 7.11 (1H, d, J=9.0 Hz), 7.35 (1H, s), 9.67 (1H, d, J=8.0 Hz), 12.49 (1H, br s)	1790, 1770, 1690
3n	1.22 (3H, t, J=7.0 Hz), 1.27 (3H, d, J=5.5 Hz), 1.36 (9H, s), 1.50 (3H, d, J=5.5 Hz), 3.50—3.80 (2H, m), 3.89 (3H, s), 3.90—4.30 (1H, m), 4.17 (2H, q, J=7.0 Hz), 5.16 (1H, d, J=5.0 Hz), 5.90 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.90 (1H, q, J=6.0 Hz), 7.13 (1H, d, J=8.0 Hz), 7.34 (1H, s), 9.68 (1H, d, J=8.0 Hz), 12.50 (1H, br s)	1790, 1770, 1690
3o	1.13 (6H, d, J=6.0 Hz), 1.24 (3H, d, J=5.5 Hz), 1.49 (9H, s), 1.53 (3H, d, J=5.5 Hz), 3.40—3.80 (2H, m), 3.94 (3H, s), 3.96—4.40 (1H, m), 4.60—5.10 (1H, m), 5.14 (1H, d, J=5.0 Hz), 5.95 (1H, dd, J=5.0, 8.0 Hz), 6.50—7.00 (2H, m), 7.20 (1H, d, J=8.0 Hz), 7.40 (1H, s), 9.74 (1H, d, J=8.0 Hz), 12.56 (1H, br s)	1790,1765, 1690
3p	1.00—2.20 (10H, m), 1.27 (3H, d, J=5.5 Hz), 1.36 (9H, s), 1.50 (3H, d, J=5.5 Hz), 3.50—3.70 (2H, m), 3.90 (3H, s), 3.90—4.30 (1H, m), 4.40—4.80 (1H, m), 5.15 (1H, d, J=5.0 Hz), 5.90 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.78 (1H, q, J=5.5 Hz), 7.11 (1H, d, J=8.0 Hz), 7.34 (1H, s), 9.67 (1H, d, J=8.0 Hz), 12.50 (1H, br s)	1795, 1765, 1690

3.90 (s, 3H), 3.90—4.30 (m, 1H), 5.16 (d, 1H, J=5.0 Hz), 5.65—6.10 (m, 3H), 6.50—6.80 (m, 1H), 7.44 (s, 1H), 8.40—8.90 (br, 3H), 9.68 (d, 1H, J=8.0 Hz), 13.0 (br, 1H). *Anal*. Calcd for $C_{22}H_{29}ClN_6O_8S_2$: C, 43.44; H, 4.86; Cl, 5.83; N, 13.82; S, 10.54. Found: C, 43.21; H, 4.82; Cl, 5.80; N, 13.82; S, 10.48.

Compounds **4g**, **4i**—**4p** were prepared by a procedure similar to that described above. IR and ^{1}H-NMR spectral data are listed in Table 7.

Diphenylmethyl 7β-[(*Z*)-2-[2-*N*-(*tert*-Butoxycarbonyl)-(*S*)-alanylaminothiazol-4-yl)]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (6b) To a solution of **5** (2.0 g) and *N*-Boc-L-alanine (1.51 g) in methylene chloride (10 ml) was added EDC·HCl (1.53 g) and DMAP (135 mg) at room temperature. After stirring for 2 h, the reaction mixture was washed with 10% aq. citric acid, 5% aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (Daisogel No. 1001 W, eluent; Benzene–EtOAc, 4 : 1→3 : 1→2 : 1) to give **6b** (1.6 g) in 61% yield. IR (Nujol) cm^{-1}: 3270, 1780, 1755, 1680. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (d, 3H, J=37.0 Hz), 1.40 (s, 9H), 3.46—3.83 (m, 2H), 3.94 (s, 3H), 4.00—4.50 (m, 1H), 5.18 (d, 1H, J=5.0 Hz), 6.01 (dd, 1H, J=5.0, 9.0 Hz), 6.54—7.04 (m, 1H), 6.94 (s, 1H), 7.04—7.89 (m, 12H), 9.76 (d, 1H, J=9.0 Hz), 12.76 (br s, 1H).

Compounds **6a**, **6c**—**6f** were prepared by a procedure similar to that described above.

7β-[(*Z*)-2-[2-(*S*)-Alanylaminothiazol-4-yl)]-2-methoxyimino-acetamido]-3-cephem-4-carboxylic Acid Hydrochloride (7b) To a solution of **6b** (1.2 g) in formic acid (7.2 ml) was added 10.1 N HCl–isopropanol (1.2 ml) at 7 °C with stirring. After stirring for 1 h at 0—5 °C, the reaction mixture was poured into diethyl ether (400 ml). The resulting precipitate was collected by filtration. The solid was dissolved in methanol and poured into diisopropyl ether to give **7b** (0.52 g) as a powder in 63% yield. IR (Nujol) cm^{-1}: 3400, 3250, 1785, 1700, 1660. ^{1}H-NMR (DMSO-d_6) δ: 1.53 (d, 3H, J=7.0 Hz), 3.66 (br s, 2H), 3.94 (s, 3H), 3.90—4.40 (m, 1H), 5.14 (d, 1H, J=5.0 Hz), 5.90 (dd, 1H, J=5.0, 9.0 Hz), 6.20—6.70 (m, 1H), 7.54 (s, 1H), 8.00—14.00 (br, 5H), 9.75 (d, 1H, J=8.0 Hz).

Compounds **7a**, **7c**—**7f** were prepared by a procedure similar to that described above. ^{1}H-NMR spectral data are listed in Table 8.

Table 7. ^{1}H-NMR and IR Spectral Data (**4g**, **4i**—**4p**)

Compound No.	^{1}H-NMR(60 MHz, DMSO-d_6, δ)	IR (Nujol) cm^{-1}
4g	1.16 (9H, s), 3.50—3.70 (2H, m), 3.70—4.10 (2H, m), 3.90 (3H, s), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.44 (1H, s), 8.40—8.80 (3H, br), 9.68 (1H, d, J=8.0 Hz)	1790, 1755, 1680
4i	0.80—1.20 (6H, m), 1.16 (9H, s), 1.80—2.50 (1H, m), 3.40—3.80 (2H, m), 3.70—4.00 (1H, m), 3.90 (3H, s), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.46 (1H, s), 8.50—9.00 (3H, br), 9.68 (1H, d, J=8.0 Hz)	1790, 1755, 1680
4j	0.80—1.10 (6H, m), 1.16 (9H, s), 1.50—1.90 (3H, m), 3.50—3.80 (2H, m), 3.90 (3H, s), 3.90—4.30 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.45 (1H, s), 8.50—9.00 (3H, br), 9.67 (1H, d, J=8.0 Hz)	1790, 1755, 1680
4k	0.70—1.10 (6H, m), 1.16 (9H, s), 1.40—2.30 (3H, m), 3.50—3.80 (2H, m), 3.80—4.10 (1H, m), 3.91 (3H, s), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.45 (1H, s), 8.50—9.00 (3H, br), 9.68 (1H, d, J=8.0 Hz)	1790, 1755, 1680
4l	1.16 (9H, s), 1.20—2.10 (6H, m), 2.60—3.00 (2H, m), 3.50—3.80 (2H, m), 3.80—4.30 (1H, m), 3.90 (3H, s), 5.16 (1H, d, J=5.0 Hz), 5.70—6.00 (3H, m), 6.50—6.70 (1H, m), 7.45 (1H, s), 8.00—8.50 (3H, br), 8.60—9.00 (3H, br), 9.68 (1H, d, J=8.0 Hz)	1790, 1755, 1675
4m	1.48 (6H, d, J=6.0 Hz), 2.05 (3H, s), 3.50—3.70 (2H, m), 3.80—4.30 (1H, m), 3.90 (3H, s), 5.15 (1H, d, J=5.0 Hz), 5.86 (1H, dd, J=5.0, 8.0 Hz), 6.40—6.70 (1H, m), 6.88 (1H, q, J=6.0 Hz), 7.45 (1H, s), 8.30—9.20 (3H, br), 9.68 (1H, d, J=8.0 Hz), 12.70—13.40 (1H, br)	1780, 1740, 1675
4n	1.23 (3H, t, J=7.0 Hz), 1.51 (6H, d, J=6.0 Hz), 3.50—3.80 (2H, m), 3.90—4.30 (1H, m), 3.91 (3H, s), 4.16 (2H, q, J=7.0 Hz), 5.16 (1H, d, J=5.0 Hz), 5.90 (1H, dd, J=5.0, 8.0 Hz), 6.50—6.70 (1H, m), 6.78 (1H, q, J=6.0 Hz), 7.45 (1H, s), 8.10—9.10 (3H, br), 9.67 (1H, d, J=8.0 Hz), 12.80—13.30 (1H, br)	1780, 1740, 1670
4o	1.28 (6H, d, J=7.0 Hz), 1.54 (6H, d, J=7.0 Hz), 3.50—3.84 (2H, m), 3.95 (3H, s), 4.00—4.40 (1H, m), 4.60—5.10 (1H, m), 5.17 (1H, d, J=5.0 Hz), 5.97 (1H, dd, J=5.0, 9.0 Hz), 5.49—7.10 (2H, m), 7.51 (1H, s), 7.90—10.20 (3H, br), 9.75 (1H, d, J=9.0 Hz), 11.80—14.00 (1H, br)	1780,1765, 1705, 1670
4p	0.96—2.20 (10H, m), 1.52 (6H, d, J=7.0 Hz), 3.66 (2H, br s), 3.93 (3H, s), 4.00—4.32 (1H, m), 4.32—4.86 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.94 (1H, dd, J=5.0, 9.0 Hz), 6.46—7.02 (2H, m), 7.46 (1H, s), 7.96—9.02 (3H, br), 9.73 (1H, d, J=9.0 Hz), 12.80—13.24 (1H, br)	1790, 1760, 1690, 1660

Table 8. ^{1}H-NMR Spectral Data (**7a**, **7c**—**7f**)

Compound No.	^{1}H-NMR (60 MHz, DMSO-d_6, δ)
7a	3.40—3.70 (2H, m), 3.70—4.10 (2H, m), 3.90 (3H, s), 4.20—8.00 (1H, br), 5.11 (1H, d, J=5.0 Hz), 5.84 (1H, dd, J=5.0, 8.0 Hz), 6.35—6.60 (1H, m), 7.44 (1H, s), 8.20—8.70 (3H, br), 9.66 (1H, d, J=8.0 Hz), 12.60—13.50 (1H, br)
7c	0.98 (6H, d, J=6.5 Hz), 1.90—2.45 (1H, m), 2.70—5.30 (1H, br), 3.40—3.70 (2H, m), 3.70—4.10 (1H, m), 3.91 (3H, s), 5.12 (1H, d, J=5.0 Hz), 5.80 (1H, dd, J=5.0, 8.0 Hz), 6.30—6.70 (1H, m), 7.46 (1H, s), 8.30—9.00 (3H, br), 9.66 (1H, d, J=8.0 Hz), 12.70—13.40 (1H, br)
7d	0.92 (6H, d, J=5.5 Hz), 1.40—2.00 (3H, m), 3.00—6.20 (1H, br), 3.50—3.75 (2H, m), 3.75—4.20 (1H, m), 3.91 (3H, s), 5.13 (1H, d, J=5.0 Hz), 5.83 (1H, dd, J=5.0, 8.0 Hz), 6.35—6.60 (1H, m), 7.48 (1H, s), 8.50—9.10 (3H, br), 9.68 (1H, d, J=8.0 Hz), 12.60—13.70 (1H, br)
7e	0.70—1.10 (6H, m), 1.40—2.30 (3H, m), 2.60—4.50 (1H, br), 3.40—3.75 (2H, m), 3.75—4.10 (1H, m), 3.90 (3H, s), 5.11 (1H, d, J=5.0 Hz), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.30—6.65 (1H, m), 7.46 (1H, s), 8.20—9.20 (3H, br), 9.66 (1H, d, J=8.0 Hz), 12.80—13.35 (1H, br)
7f	1.10—2.20 (6H, m), 2.60—2.90 (2H, m), 3.00—6.00 (1H, br), 3.40—3.75 (2H, m), 3.75—4.30 (1H, m), 3.91 (3H, s), 5.12 (1H, d, J=5.0 Hz), 5.85 (1H, dd, J=5.0, 8.0 Hz), 6.40—6.60 (1H, m), 7.46 (1H, s), 7.80—8.40 (3H, br), 8.40—9.20 (3H, br), 9.67 (1H, d, J=8.0 Hz), 12.75—13.30 (1H, br)

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